American Journal of Medical Genetics 69:102–106 (1997)

Identification of Mutations in the CACNL1A3 Gene in 13 Families of Scandinavian Origin Having Hypokalemic Periodic Paralysis and Evidence of a Founder Effect in Danish Families

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Familial hypokalemic periodic paralysis (hypoPP) is an autosomal dominant disorder characterised by episodic attacks of paralysis of varying severity. Recently, linkage was found to markers in 1q31–32 and to the gene encoding the muscle DHP-sensitive calcium channel α 1-subunit (CACNL1A3). Subsequently, three mutations in this gene were identified in patients with hypoPP: Arg528His, Arg1239His and Arg1239Gly. In this study, two different mutations were found in the CACNL1A3 gene in 13 Scandinavian families, 10 of whom have the Arg528His mutation while 3 families have the Arg1239His. Furthermore, there is evidence of a founder effect in 8 of the 9 Danish hypoPP families investigated, consisting of haplotypes of microsatellite markers close to and within the CACNL1A3 gene and of the geographic origin of the families. For the first time, reduced penetrance in males with the Arg528His mutation was found in several cases. Am. J. Med. Genet. 69:102–106, 1997.

KEY WORDS: hypokalemia; linkage; mutation; paralysis; periodic

INTRODUCTION

Familial hypokalemic periodic paralysis (hypoPP) is an autosomal dominant disease with attacks of paralysis of varying severity, accompanied by hypokalemia. The attacks occur at intervals of days, up to years, in otherwise healthy people. The onset usually occurs in the second decade of life and the prevalence is 1.7 per 100,000 births in Denmark and 0.8 per 100,000 in Finland [Kantola and Tarssanen, 1992]. The paralytic attacks can be precipitated by a variety of different factors, such as a carbohydrate-rich meal, cold exposure, exercise followed by rest, mental stress, and occasionally pregnancy or alcohol imbibition. Other symptoms are interictal muscular weakness and progressive myopathy.

HypoPP belongs to a group of familial periodic paralyses, which is subdivided into three types: normo-, hyper- and hypokalemic periodic paralysis. The hyperkalemic type of this disorder was found in 1991 to be related to an adult muscle sodium channel α-subunit (SCN4A) gene located in 17q [Fontaine et al., 1990]. Mutations in this gene have been identified [Ptácek et al., 1991; Rojas et al., 1991]. Some families have mutations in the SCN4A gene, but hyperkalemia has not been documented in the affected individuals during the paralysis attacks [Plassart et al., 1994b]. It is therefore conceivable that normo- and hyperkalemic periodic paralysis should not be regarded as distinct phenotypes.

Recently, hypoPP was linked to 1q31–32 [Fontaine et al., 1994] and the gene was mapped between D1S413 and D1S249. The calcium channel 1A3 (CACNL1A3) gene was mapped to this interval and was analysed for mutations as a candidate gene, even though it was unclear how defects in a Ca^{2+} channel could result in a phenotype with hypokalemia. The CACNL1A3 protein consists of four domains of internal homology, connected by intracellular loops. Each of these domains contains 6 hydrophobic segments (S1 to S6). Three different point mutations in the CACNL1A3 gene have been found, resulting in aminoacid substitu-

Contract grant sponsor: Swedish Medical Research Council; Contract grant sponsor: Nordic Council of Ministers; Contract grant sponsor: The Linnéa and Josef Carlsson Foundation; Contract grant sponsor: The Sävstaholm Foundation.

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Received 11 March 1996; Accepted 14 June 1996

tions. The first two mutations identified are substitutions of a highly conserved arginine, by either histidine (Arg1239His) or glycine (Arg1239Gly) in the S4 segment of domain 4 [Ptácek et al., 1994]. The third mutation causes the substitution of histidine for arginine (Arg528His) in the S4 segment of domain 2 [Jurkat-Rott et al., 1994]. Genetic heterogeneity for hypoPP has been published for one family by Plassart et al. [1994a].

In the present study, 13 families of Scandinavian origin (Danish, Finnish and Norwegian) have undergone mutation analysis regarding the three hitherto known mutations in the CACNL1A3 gene. The haplotypes of the Scandinavian families were also investigated for microsatellite markers located near the CACNL1A3 locus (D1S1726, an intragenic marker for CACNL1A3 and D1S1723) to search for a founder effect.

MATERIALS AND METHODS

Patients and Families

Analysis was made of 9 Danish, 1 Norwegian and 3 Finnish families. The Danish families were partly clinically reviewed earlier and are identified as families D1, D2, D4, D5, D6, D7 [Johnsen, 1980; Sagild and Helweg-Larsen, 1955], and D13, D19 and D21 (Sørensen and Laue Friis, unpublished data). The Finnish families were also reviewed previously and designated families F2, F4 and F6 [Kantola, 1988]. These 12 families were chosen for analysis from among 21 Danish and 7 Finnish known hypoPP families, based on the following criteria: highly reliable hypoPP history in the patients, willingness to cooperate, and patients still alive. The Norwegian family N1 has not been studied earlier. Mean age at onset in the 9 Danish families was 19 8/12 years for the men (range 6–70) and for women 22 4/12 (range 4–69). For the 3 Finnish families, the mean age at onset was 8 5/12 years (range 5.5–13) and in the Norwegian family 9 7/12 years (range 5–14). The frequency of paralytic attacks in the families ranged from weekly to a couple of attacks during the whole lifetime. The attacks have lasted for as little as 2 hours and as long as 72 hours. The male/female ratio for hypoPP in the Danish families is 2.3:1 and in the Finnish families, 1.9:1 [Kantola, 1988]. In the Norwegian family only males are affected. Informed consent was obtained from all participating individuals and the project was approved by medical ethics committees in Denmark and Sweden.

Linkage and Haplotype Analysis

A DNA analysis was made of 289 family members of the 13 families: 67 affected, 12 healthy nonpenetrant transmitting individuals, 170 unaffected siblings and 40 spouses. Genomic DNA was isolated from white blood cells, using phenol/chloroform extraction [Kunkel et al., 1977]. A genome scan using microsatellite markers was performed on 48 persons from pedigree D6 to ascertain linkage to the gene. Linkage analysis was calculated on a Sun Sparcstation 5 with the LINKAGE 5.1 program package, using age- and sex-dependent penetrance. The dinucleotide repeat polymorphisms D1S1273, D1S1276, [Weissenbach et al., 1992] and CACNL1A3 [Gregg et al., 1993] were detected after Polymerase chain reaction (PCR) amplification of genomic DNA. This was carried out in microtiterplate format in a 10 μl reaction volume, with one primer end-labelled with γ-^{32}P-dATP (3,000 Ci/mM, Dupont, Belgium), using between 1 and 3 pmol primer and 18 ng DNA. The PCR consisted of 28 cycles: 94°30″, 55°30″, 72°30″, initiated by one cycle of 4′ denaturation and ending with one 4′ final extension step. The PCR products were then separated by electrophoresis on a 6% polyacrylamide (PA) gel with 7 M UREA and the gel was fixed, dried and processed for autoradiography. Marker D1S1726 was located centromerically and marker D1S1723 telomerically to the disease locus CACNL1A3 by recombinations. The genetic distance between the flanking markers is 0 cM in 8 CEPH pedigrees [Jurkat-Rott et al., 1994].

Mutation Analysis

As the Arg528His mutation causes the loss of a BbvI site, it was easily analysed by PCR according to the protocol of Jurkat-Rott et al. [1994], using forward primer 5′-GGAGATCCTGCTGGTGGAGTCG-3′ and reverse primer 5′-TCCTCAGGAGGCGGATGCAG-3′. The PCR products were digested overnight with BbvI and then separated on a 4% NuSieve agarose gel. After ethidium bromide staining, the normal individuals show two bands of 33 and 44 bp and the affected individuals an additional band of 77 bp. The Argl239His mutation analysis was performed with PCR, with both the forward primer 5′-CGCATCTCCAGCGCCTTCTTC-3′ and the reverse primer 5′-CGTCCACAGGAGGGTTCGCAC-3′ end-labelled with γ-^{32}P-ATP. After digestion of the PCR products with NlaIII, the samples were run on a 20% polyacrylamide gel and autoradiographed o/n. Normal individuals have bands of 54 and 39 bp and as the Arg1239His mutation creates a new NlaIII restriction site in the 39 bp fragment, the affected individuals have bands of 54, 39, 34 and 5 bp.

RESULTS

During genome scanning, DNA was analysed from 48 subjects belonging to family D6, the largest Danish family. After 351 microsatellite markers had been analysed, about 70% of the genome had been excluded. Then linkage to the CACNL1A3 gene was reported and this could be confirmed in the present 13 hypoPP families. The Arg528His mutation was found in all of the 9 Danish families investigated and also in one of the families of Finnish origin, F2. Figure 1 presents the results

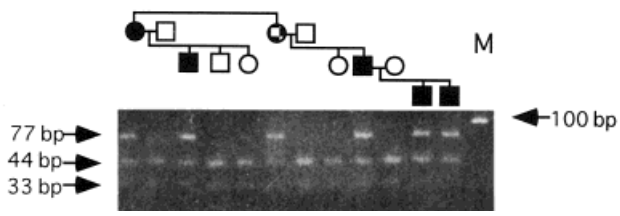

Fig. 1. Mutation analysis of a part of the Danish family D2 regarding the Arg528His mutation. The PCR products were digested with BbvI and then separated on a 4% agarose gel stained with EtBr. The mutation results in a loss of a restriction site, which is seen as an undigested band at 77 bp in affected persons. The chequered circle represents an asymptomatic female carrier and M is the DNA size marker.

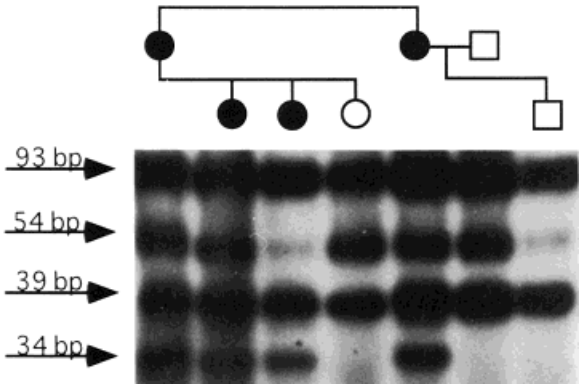

Fig. 2. Mutation analysis of the Finnish family F6 regarding the Arg1239His mutation. The products of a PCR amplification of genomic DNA and digestion with NlaIII were separated on a 20% polyacrylamide gel. The mutation creates a new restriction site for NlaIII in the 39 bp fragment, which adds an extra band of 34 bp to the digestion pattern of the affected individuals.

of the mutation analysis for a part of family D2. In Norwegian family N1 and in the Finnish families F4 and F6, the Arg1239His mutation was found to be inherited together with the hypoPP phenotype. Figure 2 shows the results of the mutation analysis for part of Finnish family F6. Haplotypes for each family are listed in Table I. Regarding the Arg528His mutation, there is evidence of a founder effect, as 8 of the 9 Danish families share the same haplotype of the three microsatellites analysed. The remaining family D1 has allele 6 segregating with the disease for the CACNL1A3 marker as the other families, but does not share the disease alleles for the flanking markers. There was no evidence of a founder effect for the Arg1239His mutation.

Incomplete penetrance in males was evident in the families with the Arg528His mutation, in addition to incomplete penetrance in women, as had been established earlier [Elbaz et al., 1995]. Figure 3 shows a part of family D6 where incomplete penetrance is present both in females and in males. Twelve healthy nonpenetrant-transmitting males were identified, 4 of whom are still alive. Two of them belong to family D6 and are presented as subjects V:13 and VI:5 in Figure 3. Subject V:13 was examined at age 58. He denied any paralytic episodes and this was confirmed by his family and his personal physician. The patient had been suffering from dementia for a couple of years, but showed no muscular atrophy. He had 1 son and 1 daughter with typical paralytic episodes; 3 daughters and 1 son were unaffected. He had 5 healthy brothers, 1 healthy sister and 2 brothers with typical paralytic episodes. One of the latter had a son, subject VI:5, who was examined at age 30 years. He denied any paralytic episode and the neurological examination was normal, though the genetic analysis revealed the mutation in the CACLN1A3 gene. Muscular biopsy was not done in either of these 2 cases.

In the Danish family material, the penetrance of the hypoPP mutation was 34.7% in females and 81.4% in males, corresponding to a male:female ratio of 2.3:1. It was found difficult to detect linkage in the Danish families, not only because of the low penetrance but also because some family members had a phenotype in many ways resembling hypoPP, but these subjects did not have the Arg528His mutation. For example, 1 female in family D2, born in 1957, suffered her first palsy attack at age 16. Her second attack was hemiparalytic at age 30 and lasted for 2 days. CT scan was normal and so was the potassium level. The subsequent attacks occurred spontaneously or were provoked by exercise, alcohol or sweets and lasted for a maximum of one hour. A glucose-provocation test proved negative, but an electromyogram, (EMG) exercise test was positive. A biopsy from the quadriceps revealed abnormalities typical of hypoPP. This female had rheumatoid arthritis diagnosed, which explains the muscular problems, and a review of the initial attacks revealed that she had been undergoing treatment with highestrogen contraceptive pills and her mother had had the same recurrent attacks during estrogen therapy. The attacks may therefore be regarded as transitory ischemic attacks. A second example is a male in family D6, born in 1930, who had had paralytic attacks from age 38 to 50. These attacks invariably occurred in the evenings. He underwent three glucose-provocation tests: in only one test did he become paralytic, but the potassium concentration did not change during the attack. When he was examined at age 62, proximal muscular atrophy of the limbs was evident. This muscular atrophy could be explained by pronounced arthrosis of the shoulders and hips, but his attacks could only be explained as psychogenic. He receives a disability pension because of the attacks.

DISCUSSION

Continuing research is at last beginning to clarify the molecular mechanism causing hypoPP. Recently a gene was mapped to 1q31–32, based on linkage analysis in three hypoPP families from different European countries. The CACNL1A3 gene was mapped to this region and subsequently mutations were identified. Heterogeneity for hypoPP has been shown for the first time in one family of French origin, viz. family D of Plassart et al. [1994a]. No difference in phenotype has been detected between this family and families with mutations in CACNL1A3.

There are certain phenotypic differences between the previously studied and the present families. The mean age at onset in the Danish families was 19–22 years, whereas it was between age 9 and 15 years in the French families A and D of Fontaine et al. [1994] and Plassart et al. [1994a] and in other Caucasian families [Elbaz et al., 1995]. There is also a reduced penetrance in the Danish families which is manifested in both males and females. Overall, the male:female ratio of the Danish affected cases is 2.3:1, viz. between 1.1:1 and 1.6:1 in families A and D. The higher age at onset in the Danish families may also have been a consequence of the lower penetrance. There has been some discussion about the lower penetrance in women, e.g., that the function or malfunction of the calcium channel may be influenced by hormone levels [Jurkatt-Rott et al., 1994; Elbaz et al., 1995]. However, this hypothesis

TABLE I. Haplotypes Segregating With the CACNL1A3 Mutations in 13 Families of Different Scandinavian Origins*

	Families with the													
	Arg528 His mutation										Arg1239 His mutation			
Locus	D1	D2	D4	D5	D6	D7	D13	D19	D21	F2	F4	F6	N1	R[a]
D1S1726	6	9	9	9	9	9	9	9	9	7	7	6	7	7,7
CACNL1A3	6	6	6	6	6	6	6	6	6	4	2	6	3	2,4
D1S1723	2	3	3	3	3	3	3	3	3	1	1	3	3	3,7

* Alleles are numbered by increasing size.
[a] Alleles of reference DNA, number 134702 from Généthon.

cannot account for the incomplete penetrance in men observed in the Danish hypoPP families. This nonpenetrance could be caused by other genes that modify the expression of the mutant gene, or by environmental influence. The high prevalence of hypoPP in Denmark may be attributed to the more extensive investigation initiated by Helweg-Larsen and Hauge in the 1940s [Helweg-Larsen et al., 1955], and continued by Johnsen in the 1970s [Johnsen, 1980]. More recently, Sørensen and Laue Friis conducted a follow-up on all published Danish families and cases retrieved from the Danish National Registry of Patients. The definition used in the Danish survey only includes patients with hypokalemic paralytic attacks, whereas in many reports, patients with myopathy without paralytic attacks have been regarded as suffering from hypoPP. However, biopsy alone is insufficient evidence of hypoPP as shown by the above-mentioned female case history.

The evidence for a founder effect in 8 of the 9 Danish families with the Arg528His mutation is based not only on the results from the haplotypes for three microsatellite markers, but also on the fact that genealogical investigations have shown that these 8 families can all be traced back to the same area in southern Jutland.

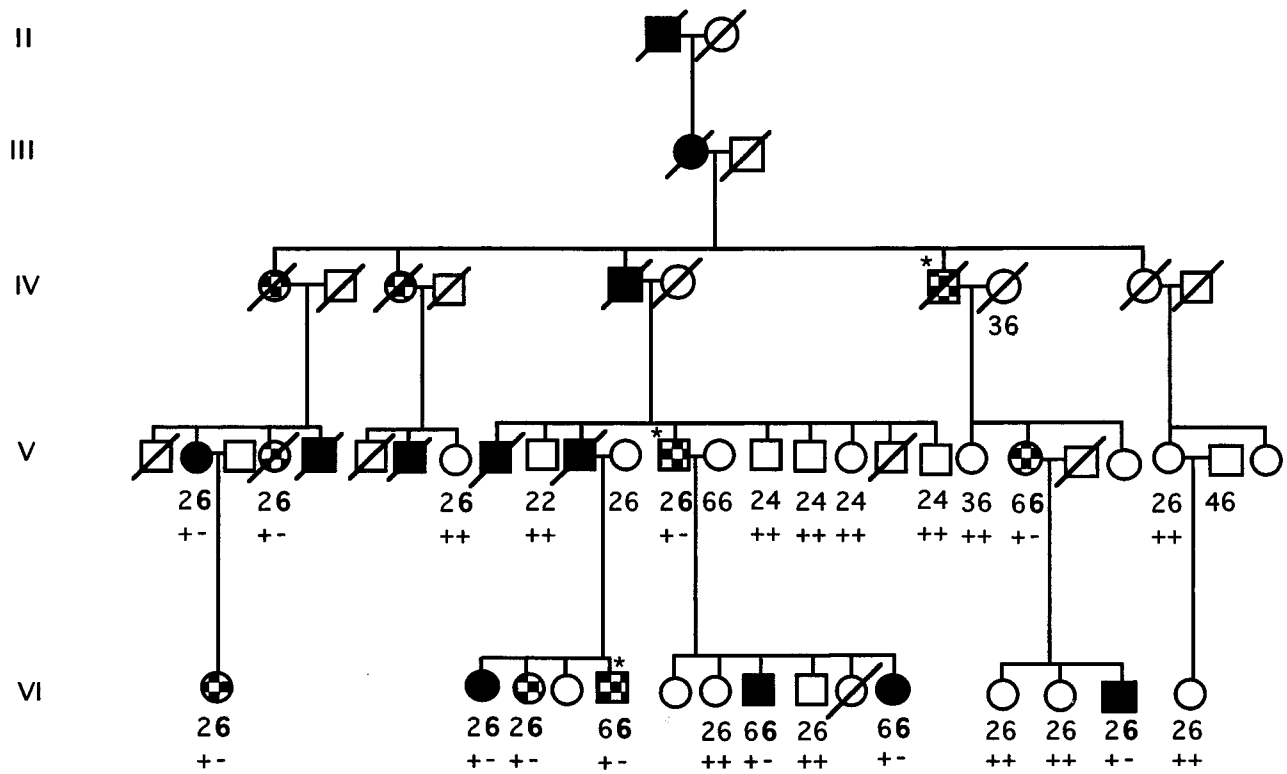

Fig. 3. Partial pedigree of the Danish family D6, showing the allele of the CACNL1A3 polymorphism segregating with the disease in bold style and mutation analysis results, where + corresponds to a normal allele and − corresponds to a mutant allele which has lost the restriction site for a BbvI site. HypoPP patients and unaffected individuals are represented, respectively, by filled and unfilled symbols and unaffected obligate hypoPP carriers are represented by chequered symbols. Individual IV:7 died at 69 years of age. Individuals V:13 and VI:5 are 61 and 31 years old, respectively.

Seven of these 8 families had ancestors living in the Esbjerg and Ribe region, in the southwestern Jutland. However, after study of old parish registers from the 18th century, there is no indication that these 8 families originate from one common ancestor. The 9th Danish family, D1, not sharing the haplotype with the other Arg528His families (except for the CACNL1A3 marker), also originates from Jutland, though from the midwest. If family D1 had originated from the same ancestor as the rest of the Danish families investigated, it might have been explained as a genetic history of a new mutation, or a crossover in both of the two flanking markers. The probability of that happening in this narrow genetic region is very small.

Transitions of CpG dinucleotides are the most common mutations [Sommer, 1995]. The average mutation rate is 3.7×10^{-8} to 1×10^{-7} per gamete per generation. This study indicates that mutations in the CpG doublet in the arginine codon has occurred on six independent occasions. It is not possible to compare the haplotypes linked to hypoPP in the present study with previous studies [Fontaine et al., 1994; Plassart et al., 1994a; Elbaz et al., 1995; Boerman et al., 1995] as allele frequencies or alleles of a reference DNA are not mentioned.

Current knowledge of the CACNL1A3 mutations also has clinical implications. In most cases the diagnosis is obvious, based on anamnesis and clinical examination. However, some of the cases presented in this study illustrate the diagnostic difficulties with hypoPP. As the present study points out, molecular analysis currently provides the opportunity for a rapid and safe method to diagnose hypoPP.

ACKNOWLEDGMENTS

We thank the family members for their cooperation, Lena Spångberg for assisting A.S. with the PCRs, and Caroline Graff for contributing with the Norwegian case material. The study was supported by the Swedish Medical Research Council, the Nordic Council of Ministers, the Linnéa and Josef Carlsson Foundation, and the Sävstaholm Foundation.

REFERENCES

Boerman RH, Ophoff RA, Links TP, van Eijk R, Sandkuijl LA, Elbaz A, Vale-Santos JE, Wintzen AR, van Deutekom JC, Isles DE, Fontaine B, Padberg GW, Frants RR (1995): Mutation in DHP receptor α 1 subunit (CACNL1A3) gene in a Dutch family with hypokalaemic periodic paralysis. J Med Genet 32:44–47.

Elbaz A, Vale-Santos J, Jurkat-Rott K, Lapie P, Ophoff RA, Bady B, Links TP, Piussan C, Vila A, Monnier N, Padberg GW, Abe K, Feingold N, Guimaraes J, Wintzen AR, van der Hoeven JH, Saudubray JM, Grunfeld JP, Lenoir G, Nivet H, Echenne B, Frants RR, Fardeu M, Lehmann-Horn F, Fontaine B (1995): Hypokalemic periodic paralysis and the dihydropyridine receptor (CACNL1A3): Genotype/phenotype correlations for two predominant mutations and evidence for the absence of a founder effect in 16 Caucasian families. Am J Hum Genet 56:374–380.

Fontaine B, Khurana T, Hoffman E, Bruns G, Haines J, Trofatter J, Hanson M, Rich J, McFairlane H, McKenna Yasek D, Romano D, Gusella J, Brown R Jr (1990): Hyperkalemic periodic paralysis and the adult muscle sodium channel gene. Science 250:1000–1002.

Fontaine B, Vale-Santos J, Jurkat-Rott K, Reboul J, Plassart E, Rime C-S, Elbaz A, Heine R, Guimaraes J, Weissenbach J, Baumann N, Fardeau M, Lehmann-Horn F (1994): Mapping of the hypokalaemic periodic paralysis (HypoPP) locus to chromosome 1q31-32 in three European families. Nature Genet 6:267–272.

Gregg RG, Couch F, Hogan K, Powers PA (1993): Assignment of the human gene for the α 1 subunit of the skeletal muscle DHP-sensitive Ca^{2+} channel (CACNL1A3) to chromosome 1q31–q32. Genomics 15:107–112.

Helweg-Larsen HF, Hauge M, Sagild U (1955): Hereditary transient muscular paralysis in Denmark. Genetic aspects of family periodic paralysis and family periodic adynamia. Acta Genet 5:263–280.

Johnsen T (1980): Familial periodic paralysis with hypokalaemia. Experimental and clinical investigations. Thesis. Lægeforeningens Forlag, Copenhagen, Denmark.

Jurkat-Rott K, Lehmann-Horn F, Elbaz A, Heine R, Gregg RG, Hogan K, Powers PA, Lapie P, Vale-Santos JE, Weissenbach J, Fontaine B (1994): A calcium channel mutation causing hypokalemic periodic paralysis. Hum Mol Genet 3:8:1415–1419.

Kantola I (1988): Familial hypokalaemic periodic paralysis in Finland—with special reference to the potassium exercise test. Thesis. Turku University, Finland.

Kantola IM, Tarssanen LT (1992): Familial hypokalaemic periodic paralysis in Finland. Neurol Neurosurg Psychiatry 55:322–324.

Kunkel L, Smith K, Boyer S, Borgaonkar D, Wachtel S, Miller O, Breg W. Jones HJ, Rary J (1977): Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants. Proc Natl Acad Sci USA 74:1245–1249.

Plassart E, Elbaz A, Vale-Santos J, Reboul J, Lapie P, Chauveau D, Jurkatt-Rott K, Guimaraes J, Saudubray J-M, Weissenbach J, Lehmann-Horn F, Fontaine B (1994a): Genetic heterogeneity in hypokalemic periodic paralysis (hypoPP). Hum Genet 94:551–556.

Plassart E, Reboul J, Rime C-S, Recan D, Millasseau P, Eymard B, Pelletier J, Thomas C, Chapon F, Desnuelle C, Confavreux C, Bady B, Martin J-J, Lenoir G, Serratrice G, Fardeau M, Fontaine B (1994b): Mutations in the muscle sodium channel gene (SCN4A) in 13 French families with hyperkalemic periodic paralysis and paramyotonia congenita: Phenotype to genotype correlations and demonstration of the predominance of two mutations. Eur J Hum Genet 2:110–124.

Ptácek L, George Jr A, Griggs R, Tawil R, Kallen R, Barchi R, Robertson M, Leppert M (1991): Identification of a mutation in the gene causing hyperkalemic periodic paralysis. Cell 67:1021–1027.

Ptácek LJ, Tawil R, Griggs RC, Engel AG, Layzer RB, Kwieci'nski H, McManis PG, Santiago L, Moore M, Fouad G, Bradley P, Leppert MF (1994): Dihydropyridine receptor mutations cause hypokalemic periodic paralysis. Cell 77:863–868.

Rojas C, Wang J, Schwartz L, Hoffman E, Powell B, Brown R Jr (1991): A Met-to-Val mutation in the skeletal muscle Na^+ channel α-subunit in hyperkalaemic periodic paralysis. Nature 354: 387–389.

Sagild U, Helweg-Larsen H (1955): Clinical features of hereditary transient paralysis. Periodic adynamia and periodic paralysis. Nordisk Medicin 53:981–985.

Sommer SS (1995): Recent human germline mutation: Inference from patients with hemophilia B. TIG 11:4:141–147.

Weissenbach J, Gyapay G, Dib C, Vignal A, Morisette J, Millasseau P, Vaysseix G, Lathrop M (1992): A second-generation linkage map of the human genome. Nature 359:794–801.